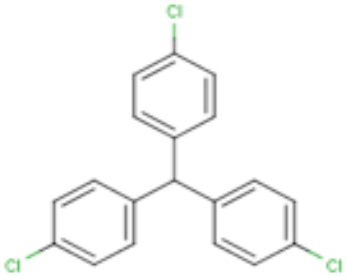
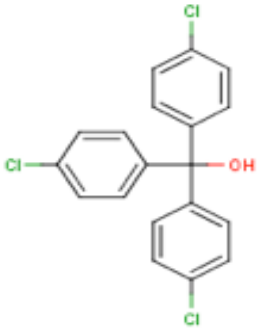
NTP Research Concept: Tris(4-chlorophenyl)methane and Tris(4-chlorophenyl)methanol



Tris(4-chlorophenyl)methane (TCPMe)



Tris(4-chlorophenyl)methanol (TCPMOH)

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Nomination Background and Rationale:
Tris(4-chlorophenyl)methane (TCPMe) is a by-product in the production of dichlorodiphenyltrichloroethane (DDT). In animal tissues where DDT was found, TCPMe and TCPMOH were also found. It is believed that the source of TCPMe is from the use of DDT and it has been assumed that TCPMOH is a metabolite of TCPMe.

TCPMe and TCPMOH were nominated by the National Institute of Environmental Health Sciences for toxicological characterization based on their widespread occurrence and persistence in the environment and limited availability of toxicity data. The compounds are presumed to be bioaccumulative and have been found in human tissues. TCPMOH is a potent competitive inhibitor of human and rodent androgen receptors *in vitro*, at concentrations within an order of magnitude as that reported in human tissues. Further studies are needed to characterize the potential human health hazard of these environmentally persistent compounds.

Both TCPMe and TCPMOH are reported to be used in the production of synthetic high polymers, lightfast dyes for acrylic fibers, and agrochemicals, indicating the potential for occupational exposure. The general population may also be exposed to TCPMe and/or TCPMOH from the consumption of food containing these compounds. Both TCPMe and TCPMOH have been found in tissues from a variety of animals, such as fish, birds, and marine mammals. TCPMe concentrations ranged from <6 µg/kg to 33,000 µg/kg lipid and the concentration of TCPMOH ranged from 13 µg/kg to 54,000 µg/kg lipid. One study reported that in human adipose, liver, bile, and breast milk the concentrations of TCPMe ranged at up to 70 ng/g lipid and that of TCPMOH at up to 38 ng/g lipid.

Oral exposure of rats to TCPMOH (1.2 mg/kg/d) for 28 days increased relative liver and spleen weights, urinary ascorbic acid, and Phase I and Phase II enzyme activities as well as changes in white blood counts. TCPMOH dose-dependently altered motility,

vitality, and acrosome reaction in human sperm *in vitro*, increased serum concentrations of follicle stimulating hormone in male rats *in vivo*, and competitively inhibited human and rat prostate androgen receptor activation *in vitro*. Information on the toxicity of TCPMe is not available. However, one study demonstrated that TCPMe and TCPMOH had mild estrogenic activity *in vitro*. Another study reported that TCPMOH was neither estrogenic nor anti-estrogenic.

Key Issues:
TCPMe and TCPMOH are accumulated in human and wildlife tissues with the highest concentration in fat. The long-term toxic effects of the chemicals are not known. Toxicological characterizations of persistent TCPMe and TCPMOH are needed for risk assessment.

Very little is known about the ADME and toxicokinetics of TCPMe and TCPMOH. No information on interaction of TCPMe and TCPMOH and/or their metabolites with macromolecules is available. Genotoxicity data are not available.

In animal studies, TCPMOH increases serum concentrations of follicle stimulating hormone in male rats, the mechanism involved is not known. In *in vitro* studies, TCPMe and TCPMOH have been reported to be estrogen mimics. TCPMOH has high affinity for androgen receptor and is a competitive antagonist of androgen receptor activation, Information on *in vivo* endocrine modulating effects and reproductive and developmental toxicity is not available. Particularly, investigations of TCPMe's and TCPMOH's effects on function and development of male and female reproductive systems are needed.

In a subchronic study, TCPMOH administration increased the relative spleen weight, induced splenic sinus hyperplasia, and increased peripheral white cell counts. Information on immunotoxicity of TCPMe and TCPMOH is not available.

Proposed Approach:

<u>Specific Aims</u>
The aims of the proposed studies are to provide data for assessing risk for human and wildlife.

Tier 1:
Specific Aim 1. Evaluate the absorption, distribution, metabolism and excretion of TCPMe and TCPMOH following oral administration to provide basis for understanding bioaccumulation and elimination kinetics of the compounds.

Since it is not known if TCPMOH is a metabolite of TCPMe, investigations of absorption, distribution, metabolism and excretion of TCPMe and TCPMOH following single and multiple exposures in rats and mice are needed. Blood and tissue levels will be evaluated at time intervals (up to 2 weeks) following the last dosing to investigate

bioaccumulation and elimination. Blood and urinary metabolites will be identified. Excretion via the bile and feces will also be noted.

Specific Aim 2. Evaluate the endocrine modulating activities of TCPMe and TCPMOH to establish their estrogenic/anti-estrogenic and androgenic/anti-androgenic activities.

In vitro studies have indicated that TCPMe and TCPMOH had mild estrogenic activity by increasing b-galactosidase activity in YRG-2 yeast cells and luciferase activity in MCF-7 human breast cancer cells. Studies will be conducted to confirm binding of TCPMe and TCPMOH to estrogen receptors *in vitro*. E2 will be used as a positive control. If a positive *in vitro* estrogenic response is obtained, this activity will be confirmed using the uterotrophic assay in rats.

In vitro studies have indicated that TCPMOH binds to androgen receptors. Studies will be conducted to confirm the binding of TCPMe and TCPMOH to androgen receptors *in vitro*. *In vivo* studies using castrated male (Hershberger screening assay) rats will be conducted to ascertain if the compounds are androgenic and/or antiandrogenic.

Specific Aim 3. Evaluate genotoxicity to understand possible interaction of TCPMe and TCPMOH or their metabolites with DNA. Mutagenicity studies in Salmonella assays and micronuclei evaluation *in vivo* will be conducted.

Tier 2:
Specific Aim 4. Investigate systemic toxicity of TCPMe and/or TCPMOH following oral exposure to identify target organs, especially after in utero exposure, effects on male and female reproductive systems since TCPMe and TCPMOH have been shown to modulate endocrine function *in vitro*.

Studies of adult (14-day and 90-day) and perinatal exposure to TCPMe and TCPMOH will be conducted to evaluate toxicity including immunotoxicity manifested in the F1 generation and to determine the dose levels for subsequent carcinogenesis studies.

Other toxic effects, including developmental immunotoxicity, will be ascertained via perinatal exposure since the compounds were found in human breast milk and data from p,p'-DDE study showed toxic effects were more pronounced in F1 generation following adult exposure.

Tier 3:
Specific Aim 5. Based on data generated in tiers 1 and 2, perinatal carcinogenesis and multigeneration continuous breeding reproduction studies will be conducted to evaluate the long-term effects of the persistent TCPMe/TCPMOH.

Significance and Expected Outcome:
TCPMe and TCPMOH are bioaccumulative and the chemicals have been identified in human tissues, but their short term and long term toxic effects are not known. The data

generated in the proposed studies will be useful in characterizing the toxic effects, in providing a basis for risk assessment, and in establishing safe exposure limits.

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